



TITLE:

Medicinal uses, thin-layer chromatography and high-performance liquid chromatography profiles of plant species from Abomey-Calavi and Dantokpa Market in the Republic of Benin

AUTHOR(S):

Dougnon, Godfried; Ito, Michiho

---

CITATION:

Dougnon, Godfried ...[et al]. Medicinal uses, thin-layer chromatography and high-performance liquid chromatography profiles of plant species from Abomey-Calavi and Dantokpa Market in the Republic of Benin. *Journal of Natural Medicines* 2020, 74(1): 311-322

ISSUE DATE:

2020-01

URL:

<http://hdl.handle.net/2433/245368>

RIGHT:

This is a post-peer-review, pre-copyedit version of an article published in 'Journal of Natural Medicines'. The final authenticated version is available online at: <http://dx.doi.org/s11418-019-01344-1>.; The full-text file will be made open to the public on 4 July 2020 in accordance with publisher's 'Terms and Conditions for Self-Archiving'; この論文は出版社版ではありません。引用の際には出版社版をご確認ご利用ください。; This is not the published version. Please cite only the published version.

**Title: Medicinal uses, thin layer chromatography and high-performance liquid chromatography profiles of plant species from Abomey-Calavi and Dantokpa Market in the Republic of Benin**

**Authors: Godfried Dougnon and Michiho Ito\***

*Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan*

\* Corresponding author:

E-mail: [michihoi@pharm.kyoto-u.ac.jp](mailto:michihoi@pharm.kyoto-u.ac.jp)

Tel: +81-75-753-4506

## Abstract

This study provides a list of popular medicinal plants found in southern Benin (West Africa) with their mode of use, diseases treated, and thin layer and high-performance liquid chromatography profiles. The list includes 10 most widely used plant species from Dantokpa Market (biggest market located in Cotonou) and Abomey-Calavi in the Republic of Benin. Species were identified by the Laboratory of Botany and Applied Ecology, University of Abomey-Calavi. Voucher specimens were deposited in the herbarium of the Experimental Station for Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University, Japan, and in the National Herbarium of Benin, University of Abomey-Calavi. The list was as follows: *Azadirachta indica* (Meliaceae), *Caesalpinia bonduc* (Caesalpiniaceae), *Catharanthus roseus* (Apocynaceae), *Garcinia kola* (Clusiaceae), *Khaya senegalensis* (Meliaceae), *Monodora myristica* (Annonaceae), *Moringa oleifera* (Moringaceae), *Talinum fruticosum* (Talinaceae), *Tridax procumbens* (Asteraceae), and *Xylopi aethiopica* (Annonaceae).

**Keywords:** Dantokpa Market, Abomey-Calavi, traditional healer, market herbalist, TLC, HPLC.

## Introduction

Africa is rich in wild plant species, many of which are used for medicinal purposes. In towns and rural villages in the Republic of Benin, traditional healers and market herbalists (called “*Amawato*” in the local language Fon) use knowledge passed on from generation to generation. According to a previous study, of the 3,000 plant species inventoried in the forests of Benin, 172 are used locally as food, while 814 are used medicinally [1]. Field surveys suggest that people who are illiterate and those living in the most remote regions use medicinal plants for primary care rather than going to a hospital [2]. There are five main reasons for this: 1) a lack of adequate health care systems or access to hospitals; 2) claims that traditional healers can cure diseases where conventional medicine has failed; 3) the greater number of traditional healers (7,500 per 10 million people) compared with medical doctors (600 per 10 million people); 4) easier access since medicinal plants are generally cultivated in home gardens and also grow wild in abundance; and 5) treatment with traditional medicine is very cheap compared with conventional medicine. Studies carried out in Abomey-Calavi and Cotonou in southern Benin demonstrated that recipes for the treatment of a disease generally vary from a combination of 2-8 medicinal plants at a cost of 300-1,000 West African CFA Franc (XOF) (60-200 Japanese yen) among market herbalists to 2,000-5,000 XOF (400-1,000 Japanese yen) among traditional healers, and these plants are often used in decoctions and infusions [2]. Due to the abundance and multiple uses of these natural resources, there is still a lot of work to do in order to report scientific data on their pharmacological effects, phytochemical composition and medicinal uses in diverse regions. We previously conducted a survey in central Benin with 174 traditional healers and 27 market herbalists and collected 410 prescriptions including more than 160 species of medicinal plant used for hypertension



[3]. In this paper, the medicinal uses, diseases treated, reported pharmacological properties, thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) profiles of 10 most widely used medicinal plants in Dantokpa Market and Abomey-Calavi (Republic of Benin) are reported. Dantokpa Market, also known as “*Tokpa*,” is the biggest open-air market in West Africa and is located in Cotonou, southern Benin.

## Materials and Methods

### Herbal material

In this study, in order to collect valuable information about the medicinal plants, 17 informants were randomly selected using purposive and snowball sampling methods [4]. Selection was based first on consultations with the president of the Association of Traditional Healers of Benin, who suggested individuals with the greatest knowledge of herbal medicine in the selected regions, and second on recommendations from Julien Djego, Professor of Botany at the School of Pharmacy, Faculty of Health Sciences, University of Abomey-Calavi. Informants were asked about the most widely used and readily available plants, their local names, uses, and diseases treated. Interviews were conducted either in Dantokpa Market or at the homes of the informants in March 2018 and a sample of each plant was bought or collected. The following plants were bought in Dantokpa Market: leaves and seeds of *Caesalpinia bonduc*, seeds of *Garcinia kola*, bark of *Khaya senegalensis*, seeds of *Monodora myristica* and fruit of *Xylopia aethiopica*. 5 plant species were collected in Abomey-Calavi: leaves and seeds of *Azadirachta indica*, leaves and roots of *Catharanthus roseus*, leaves of *Moringa oleifera*, leaves of *Talinum fruticosum* and leaves of *Tridax procumbens*. Samples were dried at ambient temperature

and identified by the Laboratory of Botany and Applied Ecology, University of Abomey-Calavi. Voucher specimens of each plant species were deposited in the herbarium of the Experimental Station for Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University, Japan, and in the National Herbarium of Benin, University of Abomey-Calavi. Registration numbers for each plant species are listed in Table 1.

### **Preparation of extracts**

Air-dried samples were ground into powder using an electric blender. 1 g powder of each sample was extracted with 10 mL 95% ethanol for 72 h under constant agitation. The obtained extracts were filtered and concentrated using a rotary evaporator. TLC and HPLC analysis were carried out as per the European Pharmacopoeia, with the purpose of acquiring chromatographic profiles of the samples [5].

### **Thin layer chromatography**

Each extract was loaded onto 4 × 7 cm pre-coated silica gel plates (TLC grade, Merck, Darmstadt, Germany) using a capillary tube, and hexane/ethyl acetate (3:1) was used as the mobile phase system. The chromatograms were observed at UV 365 nm, UV 254 nm and after staining with *p*-anisaldehyde reagent, and R<sub>f</sub> values were calculated.

### **High-performance liquid chromatography**

All chemical reagents and solvents were purchased from Nacalai Tesque Inc. (Kyoto, Japan), and were of HPLC grade or otherwise the highest grade available. Analyses were performed using an HPLC system (L-2130 pump, L-2300 column oven, L-2400 UV detector; Hitachi Ltd., Tokyo, Japan) and a 150 × 4.6 mm Cosmosil Cholesterol column (Nacalai Tesque Inc.). Ethanolic extracts were dried over anhydrous sulfate sodium and

filtered through a 0.45- $\mu$ m filter (Cosmonice Filter W, Nakalai Tesque Inc). A 10- $\mu$ L sample of each filtered extract was injected onto the column, which was maintained at 25°C. The mobile phase was composed of water and acetonitrile, and was delivered at a flow rate of 1 mL/min. Total run time was 30 min; detection was carried out at 260 nm. Following recommendations of the European Pharmacopoeia [5], the acetonitrile:water ratio was as follows: 25:75 (*A. indica* leaves, *K. senegalensis* bark), 60:40 (*A. indica* seeds, *C. roseus* leaves and roots, *X. aethiopica* fruits), 20:80 (*M. oleifera* leaves, *M. myristica* seeds), 40:60 (*T. fruticosum* leaves, *C. bonduc* leaves, *T. procumbens* leaves, *G. kola* seeds), and 90:10 (*C. bonduc* seeds).

## Results and Discussion

Data obtained in the local language were compared and cross-referenced with existing flora keys [6] to confirm authenticity. Plant species are listed in Table 1 in alphabetical order of their scientific names, with family and voucher numbers, local names, collection/purchase location, reported pharmacological effects, parts used, preparation methods, administration route, and diseases/symptoms treated in the Republic of Benin. Fig. 1 and 2 show respectively the TLC and HPLC chromatograms of the described plant species. A map representing the study area and pictures of the plant species are available as online supplementary data alongside the TLC chromatograms observed under UV 365 nm and 254 nm.

### *Azadirachta indica* (Fig. 1 and 2; 1L and 1S)

In our TLC chromatograms, similar  $R_f$  values were found for seeds and leaves of *A. indica* at 0.61 and 0.42. Azadirachtin, nimbin, quercetin and salannin are the most reported compounds of *A. indica* [7] and using hexane/ethyl acetate (1:1) as mobile phase,

nimbin and azadirachtin were previously identified with high R<sub>f</sub> values [8]. Thus, the different R<sub>f</sub> values obtained in the present study could correspond to azadirachtin, nimbin, quercetin or salannin. The HPLC chromatograms showed a variety of peaks; however, further experiments are now needed to confirm identify of these components.

#### ***Caesalpinia bonduc* (Fig. 1 and 2; 2L and 2S)**

In our TLC chromatograms, seeds and leaves of *C. bonduc* revealed similar spots at R<sub>f</sub> values of 0.84 and 0.5. The most commonly reported compounds of *C. bonduc* are bonducellin and caesalpin [9]. Our HPLC chromatograms showed few peaks whose identity need to be confirmed by further advanced phytochemical analysis and comparison with reference standards. Compounds with similar peaks and various concentrations have been previously reported in samples of the genus *Caesalpinia* [10].

#### ***Catharanthus roseus* (Fig. 1 and 2; 3L and 3R)**

At R<sub>f</sub> values of 0.44 and 0.84, same spots were revealed for roots and leaves of *C. roseus*. However, fewer spots and different R<sub>f</sub> values were observed compared with a previous study [11], possibly due to variability in the plant collection area, plant part studied, extraction method, or mobile phase system used for TLC. Few peaks were observed in our HPLC chromatograms. Vincristine, serpentine, and vinblastine have previously been identified in *C. roseus* [12], suggesting that the peaks and spots observed in this study could correspond to either of these compounds.

#### ***Garcinia kola* (Fig. 1 and 2; 4)**

R<sub>f</sub> values of 0.87 and 0.88 were previously obtained by Seanego et al. [13] using a chloroform: ethyl acetate: formic acid (10:8:2) solvent system. In the present study, we obtained only one spot at 0.65 using hexane/ethyl acetate (3:1) solvent system. This difference can be explained by variation in sample collection area, solvent system used,

or method of preparation of the samples. Our HPLC chromatogram of *G. kola* revealed two main peaks at 4.30 and 5.50 min. Phytochemical compounds such as bioflavonoids, xanthenes, and benzophenones have been isolated from the seeds of *G. kola* [14], suggesting that the spots and peaks in this study could correspond to any of these chemicals.

#### ***Khaya senegalensis* (Fig. 1 and 2; 5)**

TLC results in this study showed one to two spots from the bark of *K. senegalensis*. Previous phytochemical analysis of *K. senegalensis* reported the presence of phenols, tannins, saponins, and alkaloids using various solvent extracts [15]. Limonoids such as khayanolide A, 1 $\alpha$ ,3 $\alpha$ ,7 $\alpha$ -trideacetylkhivorin, and khayanone were previously identified from the bark of *K. senegalensis* [16]. Our HPLC chromatograms showed various peaks with different concentrations, and therefore, additional chromatographic techniques are now needed to confirm the identity of these components.

#### ***Monodora myristica* (Fig. 1 and 2; 6)**

Our TLC plates revealed several spots for the seeds of *M. myristica* using hexane/ethyl acetate (3:1). HPLC analysis showed different peaks, which could correspond to flavonoids, tannins, or glycosides as previously reported [17].

#### ***Moringa oleifera* (Fig. 1 and 2; 7)**

Our TLC results revealed several spots using hexane/ethyl acetate (3:1) with R<sub>f</sub> values from 0.4 to 0.83. Using a different solvent system, Bueno et al. [18] obtained two spots from methanolic extracts of leaves of *M. oleifera* from the Philippines. This plant reportedly contains flavonoids and phenolic compounds such as quercetin and kaempferol, and negligible amount of alkaloids [18, 19]. The different peaks obtained in

the HPLC chromatograms give an indication of their polarity, suggesting the presence of polar and non-polar compounds.

#### ***Talinum fruticosum* (Fig. 1 and 2; 8)**

Very little attention has been given to the phytochemical screening of this plant. It has been mainly reported phenolic compounds such as luteoline in the leaves of *T. fruticosum* [20]. Our TLC chromatograms showed multiple spots with R<sub>f</sub> values from 0.43 to 0.84. Our HPLC system showed few peaks; thus, additional chromatographic techniques are now needed to identify the different phytochemicals from our sample.

#### ***Tridax procumbens* (Fig. 1 and 2; 9)**

Our TLC results showed 3 spots with R<sub>f</sub> values from 0.45 to 0.82 for *T. procumbens* leaves suggesting the presence of more and less polar components. Using a different solvent system, Nisha et al. [21] have reported few spots for the leaves of *T. procumbens*. Our HPLC chromatogram showed a few peaks whose identity need to be confirmed by further advanced chromatographic techniques.

#### ***Xylopia aethiopica* (Fig. 1 and 2; 10)**

Our TLC chromatogram showed the presence of more or less polar compounds. The fruit of *X. aethiopica* has been reported to contain kaurene-type diterpenoid acids such as kaurenoic and xylopic acid [22]. These polar compounds may correspond to the lowest R<sub>f</sub> values obtained in our TLC plates. Different peaks were observed with the main one at 2.5 min in our HPLC chromatogram; therefore, further analyses are now needed to identify the different compounds.

In the present study, the leaves accounted for half of our samples and generally showed more spots on TLC plates and more peaks on HPLC chromatograms than the seeds or roots. Buhian et al. [23] presented the chromatographic fingerprints of leaves and stems

of *Muntingia calabura*, and reported that the leaves showed more spots compared to the stems. The fact that the leaves are rich in various metabolites could explain why this part of plants is preferentially used in traditional medicine, as reported previously [2, 3, 24]. To confirm the identity and isolate the various phytochemicals obtained from our plant extracts, comparative TLC experiments and additional HPLC and nuclear magnetic resonance (NMR) analyses using reference standards will be conducted in a separate study. Multiple compounds exist throughout plants, and TLC and HPLC profiling is a good first step toward determining their chemical constituents. In this study, medicinal uses, TLC and HPLC profiling data of selected plant species from southern Benin were presented for the first time.

## Conclusions

This paper focused on the medicinal uses and characterization of 10 most widely used medicinal plants from Dantokpa Market and Abomey-Calavi in the Republic of Benin. However, numerous resources exist in other markets and regions, and should also be examined in future studies. The TLC and HPLC data of the described plant species demonstrate the presence of a large range of secondary metabolites. Moreover, the various uses of these medicinal plants indicate the abundance of knowledge among traditional healers and market herbalists. This is a preliminary study and will be followed by more detailed analyses of the described species, with evaluation of their pharmacological effects.

**Funding:** None.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

## References

1. Codjia JTC, Assogbadjo AE, Ekué MRM (2003) Diversity and local valorization of Benin's food forest plant resources. *Cah Agric* 12:321–331
2. Koudokpon H, Dougnon TV, Bankolé HS, et al (2017) Ethnobotanical survey of plants used in the treatment of infections in southern Benin. *Health Sci Diseases* 18:55–71
3. Dougnon GT (2013) Ethnobotanical and ethnopharmacological studies of plants used in the traditional treatment of high blood pressure in central Benin. Thesis for the Grade of Doctor in Pharmacy, University of Abomey-Calavi, Faculty of Health Sciences. DOI: 10.13140/RG.2.2.14520.19202/1
4. Tongco MDC (2007) Purposive sampling as a tool for informant selection. *Ethnobotany Research and Applications* 5:147–158
5. European Directorate for the Quality of Medicines and Healthcare (2017) European Pharmacopoeia 9.0. Council of Europe, Strasbourg Codex, France (<http://online6.edqm.eu/ep900/>)
6. de Souza S (1988) Flora of Benin: Names of plants in Benin national languages. National University of Benin, Republic of Benin
7. Alzohairy MA (2016) Therapeutics role of *Azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. *J Evid Based Complementary Altern Med* 2016:1–11. <https://doi.org/10.1155/2016/7382506>



8. Vani MM, Rao PSS, Varma GN, et al (2016) Identification and chemical characterization of *Azadirachta indica* leaf extracts through thin layer chromatography. *Int J Res Eng Technol* 5:117–122
9. Schmelzer GH, Gurib-Fakim A (2008) Plant Resources of Tropical Africa (PROTA) Volume 11. Medicinal Plants 1. PROTA Foundation, Wageningen, Netherlands
10. Parveen A, Zahra Z, Farooqi MQ, et al (2017) Phytochemical screening and content determination of different species of genus *Caesalpinia* belonging to different origin with antidiabetic activity. *Pharmacogn J* 9:743–749.  
<https://doi.org/10.5530/pj.2017.6.117>
11. Kabesh K, Senthilkumar P, Rangunathan R, Kumar RR (2015) Phytochemical analysis of *Catharanthus roseus* plant extract and its antimicrobial activity. *Int J Pure Appl Biosci* 3:162–72
12. Tikhomiroff C, Jolicoeur M (2002) Screening of *Catharanthus roseus* secondary metabolites by high-performance liquid chromatography. *J Chromatogr* 955:87–93.  
[https://doi.org/10.1016/S0021-9673\(02\)00204-2](https://doi.org/10.1016/S0021-9673(02)00204-2)
13. Seanego CT, Ndip RN (2012) Identification and antibacterial evaluation of bioactive compounds from *Garcinia kola* (Heckel) seeds. *Molecules* 17:6569–6584.  
<https://doi.org/10.3390/molecules17066569>
14. Tshibangu PT, Kapepula PM, Kapinga MJK, et al (2016) Fingerprinting and validation of a LC-DAD method for the analysis of biflavanones in *Garcinia kola*-based antimalarial improved traditional medicines. *J Pharm Biomed Anal* 128:382–390. <https://doi.org/10.1016/j.jpba.2016.04.042>

15. Sani AA, Alemika TE, Zakama S, et al (2013) Phytochemical screening and thin layer chromatography of the leaves of *Khaya senegalensis* (dry zone mahogany) Meliaceae. J Pham Bioresour 9:20–23. <https://doi.org/10.4314/jpb.v9i1.4>
16. Zhang H (2008) Characterization of bioactive phytochemicals from the stem bark of African Mahogany *Khaya senegalensis* (Meliaceae). Dissertation for the Grade of Doctor of Philosophy in Food Technology, Clemson University, South Carolina, USA ([https://tigerprints.clemson.edu/all\\_dissertations/305](https://tigerprints.clemson.edu/all_dissertations/305))
17. Moukette BM, Pieme CA, Njimou JR, et al (2015) In vitro antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as spice: *Monodora myristica*. Biol Res 48:1–17. <https://doi.org/10.1186/s40659-015-0003-1>
18. Bueno PR, Alvarez MR, Cruz RO, et al (2016) Thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) profiling and phytochemical analysis of *Euphorbia hirta*, *Gliricidia sepium* and *Moringa oleifera* methanol extracts. Der Pharma Chem 8:456–461
19. Leone A, Fiorillo G, Criscuoli F, et al (2015) Nutritional characterization and phenolic profiling of *Moringa oleifera* leaves grown in Chad, Sahrawi refugee camps, and Haiti. Int J Mol Sci 16:18923–18937. <https://doi.org/10.3390/ijms160818923>
20. Kiranmayee BR, Sirisha GVD, Rachel VK, Jha A (2016) Structural characterization and evaluation of antioxidant activity of isolated phenolic compounds from *Talinum fruticosum* leaves. Int J Pharm Sci Rev Res 38:75–82

21. Nisha MH (2010) Phytochemical and biological investigation of *Tridax procumbens* leaves. Thesis for degree of B. Pham, East West University, Aftabnagar, Dhaka of Bangladesh (<http://dspace.ewubd.edu/handle/123456789/1691>)
22. Ekong DEU, Ogan AU (1968) Chemistry of the constituents of *Xylopi aethiopica*. The structure of xylopic acid, a new diterpene acid. J Chem Soc Chem 69:311–312. <https://doi.org/10.1039/j39680000311>
23. Buhian WPC, Rubio RO, Martin-Puzon JJ (2017) Chromatographic fingerprinting and free-radical scavenging activity of ethanol extracts of *Muntingia calabura* L. leaves and stems. Asian Pac J Trop Biomed 7:139–143. <https://doi.org/10.1016/j.apjtb.2016.11.016>
24. Fah L, Klotoé JR, Dougnon V, et al (2013) Ethnobotanical study of plants used in the treatment of diabetes in pregnant women in Cotonou and Abomey - Calavi (Benin). J Anim Plant Sci 18:2647–2658
25. Adepoju AO, Ogunkunle ATJ, Femi-Adepoju AG (2014) Antifungal activities of seed oil of neem (*Azadirachta indica* A. Juss). Glob J Biol Agr Heath Sci 3:106–109
26. Ospina Salazar DI, Hoyos Sánchez RA, Orozco Sánchez F, et al (2015) Antifungal activity of neem (*Azadirachta indica*: Meliaceae) extracts against dermatophytes. Acta Biol Colomb 20:201–207. <https://doi.org/10.15446/abc.v20n3.45225>
27. Bharat P, Sagar R, Sulav R, Ankit P (2015) Investigations of antioxidant and antibacterial activity of leaf extracts of *Azadirachta indica*. Afr J Biotechnol 14:3159–3163. <https://doi.org/10.5897/AJB2015.14811>

28. Farahna M, Bedri S, Khalid S, et al (2010) Anti-plasmodial effects of *Azadirachta indica* in experimental cerebral malaria: Apoptosis of cerebellar Purkinje cells of mice as a marker. North Am J Med Sci 518–525.  
<https://doi.org/10.4297/najms.2010.2518>
29. Noorani AA, Gupta K, Bhadada K, Kale MK (2011) Protective effect of methanolic leaf extract of *Caesalpinia bonduc* (L.) on gentamicin-induced hepatotoxicity and nephrotoxicity in rats. Iran J Pharm Ther 10:21–25
30. Gupta M, Muzumder U, Kumar R (2004) Antitumor activity and antioxidant status of *Caesalpinia bonducella* against ehrlich ascites carcinoma in swiss albino mice. J Pharmacol Sci 94:177–184
31. Kumar RS, Kumar KA, Murthy NV (2010) Hepatoprotective and antioxidant effects of *Caesalpinia bonducella* on carbon tetrachloride-induced liver injury in rats. Int J Plant Sci 1:062–068
32. Tiong S, Looi C, Hazni H, et al (2013) Antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L.) G. Don. Molecules 18:9770–9784.  
<https://doi.org/10.3390/molecules18089770>
33. Nayak BS, Pinto PLM (2006) *Catharanthus roseus* flower extract has wound-healing activity in Sprague Dawley rats. BMC Complement Altern Med 6:1–6
34. Akoachere TK, Ndip RN, Chenwi EB, et al (2002) Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens. East Afr Med J 79:588–592

35. Paul CA, Fabian OC, Okechukwu PCU (2014) Antimicrobial effects of bitter kola (*Garcinia kola*) nut on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. IOSR J Dent Med Sci 13:29–32. <https://doi.org/10.9790/0853-13452932>
36. Zhang H, Wang X, Chen F, et al (2007) Anticancer activity of limonoid from *Khaya senegalensis*. Phytother Res 21:731–734
37. Tatsadjieu LN, Essia-Ngang JJ, Ngassoum MB, Etoa FX (2003) Antibacterial and antifungal activity of *Xylopi aethiopica*, *Monodora myristica*, *Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon. Fitoterapia 74:469–472. [https://doi.org/10.1016/S0367-326X\(03\)00067-4](https://doi.org/10.1016/S0367-326X(03)00067-4)
38. Gupta R, Mathur M, Bajaj VK, et al (2012) Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. J Diabetes 4:164–171
39. Elgamily H, Moussa A, Elboraey A, et al (2016) Microbiological assessment of *Moringa oleifera* extracts and its incorporation in novel dental remedies against some oral pathogens. Open Access Maced J Med Sci 4:585–590
40. Toppo R, Roy BK, Gora RH, et al (2015) Hepatoprotective activity of *Moringa oleifera* against cadmium toxicity in rats. Vet World 8:537–540
41. Nikkon F, Saud ZA, Rahman MH, Haque ME (2003) In vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. Pak J Biol Sci 6:1888–1890

42. Liao DY, Chai YC, Wang SH, et al (2015) Antioxidant activities and contents of flavonoids and phenolic acids of *Talinum triangulare* extracts and their immunomodulatory effects. J Food Drug Anal 23:294–302.  
<https://doi.org/10.1016/j.jfda.2014.07.010>
43. Olorunnisola OS, Adetutu A, Afolayan AJ, Owoade AO (2016) Effect of methanolic leaf extract of *Talinum triangulare* (Jacq). Willd. on biochemical parameters in diet induced dyslipidemia Wistar rats. Pharmacogn Mag 12:333–339.  
<https://doi.org/10.4103/0973-1296.192194>
44. Ravindra Babu (2012) Hypoglycemic activity of methanolic extract of *Talinum triangulare* leaves in normal and streptozotocin induced diabetic rats. J App Pharm Sci 2:197-201. <https://doi.org/10.7324/JAPS.2012.2537>
45. Ravikumar V, Kanchi SS, Devaki T (2005) Effect of *Tridax procumbens* on liver antioxidant defense system during lipopolysaccharide-induced hepatitis in D-galactosamine sensitised rats. Mol Cell Biochem 269:131–136
46. Ravikumar V, Shivashangari KS, Devaki T (2005) Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. J Ethnopharmacol 101:55–60
47. Asekun OT, Adeniyi BA (2004) Antimicrobial and cytotoxic activities of the fruit essential oil of *Xylopia aethiopica* from Nigeria. Fitoterapia 75:368–370.  
<https://doi.org/10.1016/j.fitote.2003.12.020>

Table 1: Medicinal plants cited in this study: scientific names, collected parts and locations, local names, pharmacological effects, part(s) used, method of preparation, administration route and disease(s) treated.

Family, Scientific name, Voucher number	Collected part; Purchase/collection location	Local name (Fon)	Pharmacological effects	Part(s) used and preparation method	Administration route	Disease(s) treated
Meliaceae, <i>Azadirachta</i> <i>indica</i> A. Juss., EST-5025 AA 6789/HNB	<b>Leaves and Seeds;</b> collected in <b>Abomey-Calavi</b>	KININUTIN	<b>Seeds:</b> antifungal [25]  <b>Leaves:</b> antifungal [26], antioxidant and antibacterial [27], anti-plasmodial [28]	<b>Leaves</b> are malaxed into balls and sun-dried, after which the balls are chewed and swallowed. An oil is made from the <b>seeds</b> , mixed with coconut oil and applied to the body An aqueous decoction of the <b>leaves</b> is mixed with the leaves of <i>Cassia siamea</i> and drunk. A decoction of <b>leaves</b> and <b>bark</b> is drunk 2-3 times a day (the preparation is very bitter). The <b>leaves</b> are reduced to a paste and chewed. Fermented sap created with the <b>bark</b> is applied to lesions. An infusion of <b>fresh leaves</b> is drunk daily.	Oral  Dermal  Oral  Oral  Dermal  Oral	Diabetes  Malaria  Ascariis  Malaria  Epilepsy  Leprosy  Hepatitis
Caesalpiniaceae, <i>Caesalpinia</i> <i>bonduc</i> (L.) <b>Roxb.,</b>	<b>Leaves and Seeds;</b> bought in <b>Dantokpa Market</b>	AJIKUN	<b>Leaves:</b> hepatoprotective and nephroprotective [29], antitumor and antioxidant [30]	A decoction of <b>roots</b> is mixed with sugar and drunk 2 hours before sexual intercourse. <b>Leaves</b> are mixed with those of <i>Bambusa</i> <i>vulgaris</i> and soaked in hot water. Two tablespoons of the cooled solution is drunk daily.	Oral  Oral	Sexual asthenia, impotence  Measles

EST-5031				Two glasses of a <b>decoction</b> of leaves and <b>roots</b> is taken daily for 7 days.	Oral	Fever and headaches
AA 6795/HNB			<b>Seeds:</b> hepatoprotective and antioxidant [31]	A hot decoction of <b>leaves</b> is massaged into the chest.	Dermal	Chest pain
				A decoction of <b>leaves</b> and <b>roots</b> is drunk during menstruation.	Oral	Painful menstruation
				Powdered <b>seed</b> is taken daily as a food supplement.	Oral	Diabetes
Apocynaceae, <i>Catharanthus roseus</i> (L.) G. Don,	<b>Leaves and Roots;</b> collected in <b>Abomey-Calavi</b>	FLAWE	<b>Leaves:</b> antidiabetic and antioxidant [32]	Thirty grams of the <b>leaves</b> is boiled in 1 L of water and drunk throughout a full day.	Oral	Diabetes
EST-5026			<b>Flowers:</b> wound healing activity [33]	Fifteen grams of <b>roots</b> is collected, washed, dried for a few days in the sun and pounded into a powder. The powder is boiled in 2 L of water, reduced to 1.5 L, cooled, and filtered. A bamboo cup of the solution is drunk in the morning on an empty stomach.	Oral	Hypertension
AA 6790/HNB				The <b>roots, stems, or leaves</b> are crushed and mixed with hot or cold water. One teaspoon (5 mL) of the infusion is drunk 2 times a day until the symptoms cease.	Oral	Diarrhea
				The <b>roots</b> are boiled with water for 5-20 min. One cup of the extract is taken orally three times a day.	Oral	Gonorrhea
Clusiaceae, <i>Garcinia kola</i> Heckel,	<b>Seeds;</b> bought in <b>Dantokpa Market</b>	AHOWE	<b>Seeds:</b> antibacterial [34], antimicrobial [35]	The <b>seeds</b> are pounded and eaten or chewed daily (bitter).	Oral	Cough Diabetes Menstrual pain
EST-5035				The <b>seeds</b> are pounded, mixed with alcohol, and the mixture is drunk.	Oral	Jaundice Anemia
AA 6799/HNB				The <b>bark</b> is soaked in palm wine and drunk.	Oral	Diarrhea



				Three <b>seeds</b> are eaten daily for 3 consecutive days (forbidden foods during treatment: lemons, oranges, alcoholic drinks, oil, pepper).	Oral	Heart palpitations and vertigo
				The <b>fruit</b> , in association with <i>Amaranthus spinosus</i> and rhizomes of <i>Zingiber officinale</i> , is ground and eaten.	Oral	Impotence
Meliaceae, <i>Khaya</i> <i>senegalensis</i> (Desr.) A. Juss., EST-5027 AA 6791/HNB	<b>Bark</b> ; bought in <b>Dantokpa Market</b>	ZUNZATIN	<b>Bark:</b> anticancer [36], antitumor and antioxidant [16]	The <b>bark</b> is boiled with water and a 30-mL glass (“ <i>talokpemi</i> ” in local language Fon) is drunk twice a day.  A decoction of <b>bark</b> is drunk.  An aqueous decoction of <b>stems</b> , <b>leaves</b> , or <b>bark</b> is used for washing or sprayed onto wounds.  One teaspoon of <b>bark</b> powder is taken daily with a meal.	Oral   Oral Dermal  Oral	Diarrhea Obesity Menstrual pain Malaria and fever Circumcision wounds Diabetes
Annonaceae, <i>Monodora</i> <i>myristica</i> (Gaertn.) Dunal, EST-5029 AA 6793/HNB	<b>Seeds</b> ; bought in <b>Dantokpa Market</b>	SASALIKUN	<b>Leaves and Bark:</b> antioxidant and free radical scavenging activities [17]  <b>Seeds:</b> antibacterial and antifungal [37]	The <b>leaves</b> are boiled with water, in association with the rhizomes of <i>Zingiber officinale</i> , and drunk.  Thirty <b>seeds</b> are macerated in 2 L of water for 2 days, then filtered and collected (~1.5 L). A bamboo cup of the solution is drunk once a day. Alternatively, 3-4 seeds are chewed and swallowed with water.  A decoction of the <b>fruit</b> and <b>root</b> is drunk daily.  A decoction of 1 kg of <b>stem bark</b> is soaked in 4 L of water for 20 min and taken every 2 days.	Oral  Oral   Oral Oral	Cough  Hypertension   Cyst and myomas Malaria
	<b>Leaves</b> ; collected in Abomey-Calavi	KPATIMATIN	<b>Pods:</b> antidiabetic and antioxidant [38]	Massage is performed using crushed <b>roots</b> .	Dermal	Rheumatism and joint pain

Moringaceae, <i>Moringa</i> <i>oleifera</i> Lam., EST-5032 AA 6796/HNB			<b>Leaves:</b> antibacterial and antifungal [39], hepatoprotective [40]	The <b>bark stem, trunk, and pulp</b> are applied locally to the forehead.	Dermal	Amnesia
				A decoction of the <b>leaves</b> is drunk three times a day.	Oral	Hypertension
				The <b>leaves</b> are usually cooked to make a sauce accompanied by corn dough.	Oral	Diabetes
			<b>Roots barks:</b> antimicrobial [41]	Lightly heated <b>leaves</b> are used to treat influenza. Cooked for longer, they are nutritious, refreshing, and slightly analgesic.	Oral	Fever and pain
				The juice of <b>fresh leaves</b> or crushed <b>root</b> is used as a revulsive.	Oral	Bronchopulmonary disorders
				The <b>bark</b> and crushed <b>leaves</b> are applied to the head.	Dermal	Migraine
				A methanolic extract of <b>leaves</b> is drunk in the morning and evening.	Oral	Anxiety
Talinaceae, <i>Talinum</i> <i>fruticosum</i> (L.) Juss., EST-5033 AA 6797/HNB	<b>Leaves;</b> collected in Abomey-Calavi	GLASEMA	<b>Leaves:</b> antioxidant [42], anti-hypercholesterolemia and antioxidant [43], hypoglycemic [44]	A decoction of <b>roots</b> is drunk three times a day for 7 days.	Oral	Hypertension
				<b>Fresh leaves</b> are crushed with leaves of <i>Vitellaria paradoxa</i> and <i>Elaeis guineensis</i> and applied to lesions.	Dermal	Furuncle
				A decoction of <b>leafy stem</b> is drunk.	Oral	Malaria
				The <b>leaves</b> are macerated in water and applied to lesions.	Dermal	Scabies
				The <b>whole plant</b> is dried and burned, formed into a powder, and taken from the second day of menstruation.	Oral	Female infertility, fibroma
		WENMI		One glass of macerated <b>leaves</b> is drunk daily.	Oral	Diarrhea

Asteraceae, <i>Tridax procumbens</i> L., EST-5034 AA 6798/HNB	<b>Leaves</b> ; collected in <b>Abomey-Calavi</b>	<b>Leaves:</b> antioxidant, anti-inflammatory and hepatoprotective [45, 46]	An aqueous decoction of the <b>whole plant</b> , in association with leaves of <i>Euphorbia hirta</i> , is drunk.	Oral	Hypertension
			An aqueous decoction of <b>leaves</b> with flowers of <i>Phyllanthus amarus</i> and <i>Alternanthera sessilis</i> is drunk	Oral	Amenorrhea
			The <b>leafy stem</b> and <b>pulp</b> are crushed and applied to lesions.	Dermal	Edema
Annonaceae, <i>Xylopia aethiopica</i> (Dunal) A. Rich, EST-5030 AA 6794/HNB	<b>Fruit</b> ; bought in <b>Dantokpa Market</b>	KPEJELEKUN <b>Fruit:</b> antibacterial and antifungal [37], antimicrobial and cytotoxic [47]	The <b>fruit</b> is washed with the fruit of <i>Tetrapleura tetraptera</i> , <i>Crinum jagus</i> bulb, and the roots of <i>Securidaca longepedunculata</i> , cut into pieces, soaked in water for three days and drunk (adults: 1 small glass daily; children: 1 teaspoon daily).	Oral	Asthma
			A decoction of <b>leaves</b> with roots of <i>Cocos nucifera</i> is drunk daily.	Oral	Fibroma
			The <b>fruit</b> is chewed (seeds are used in rituals, in association with <i>Schrebera arborea</i> , <i>Afzelia africana</i> , and <i>Tetrapleura tetraptera</i> ).	Oral	Anxiety
			Ripe <b>fruit</b> is mixed with aerial parts of <i>Solanum nigrum</i> , reduced to a powder, and applied to lesions.	Dermal	Wounds

## Figure Legends

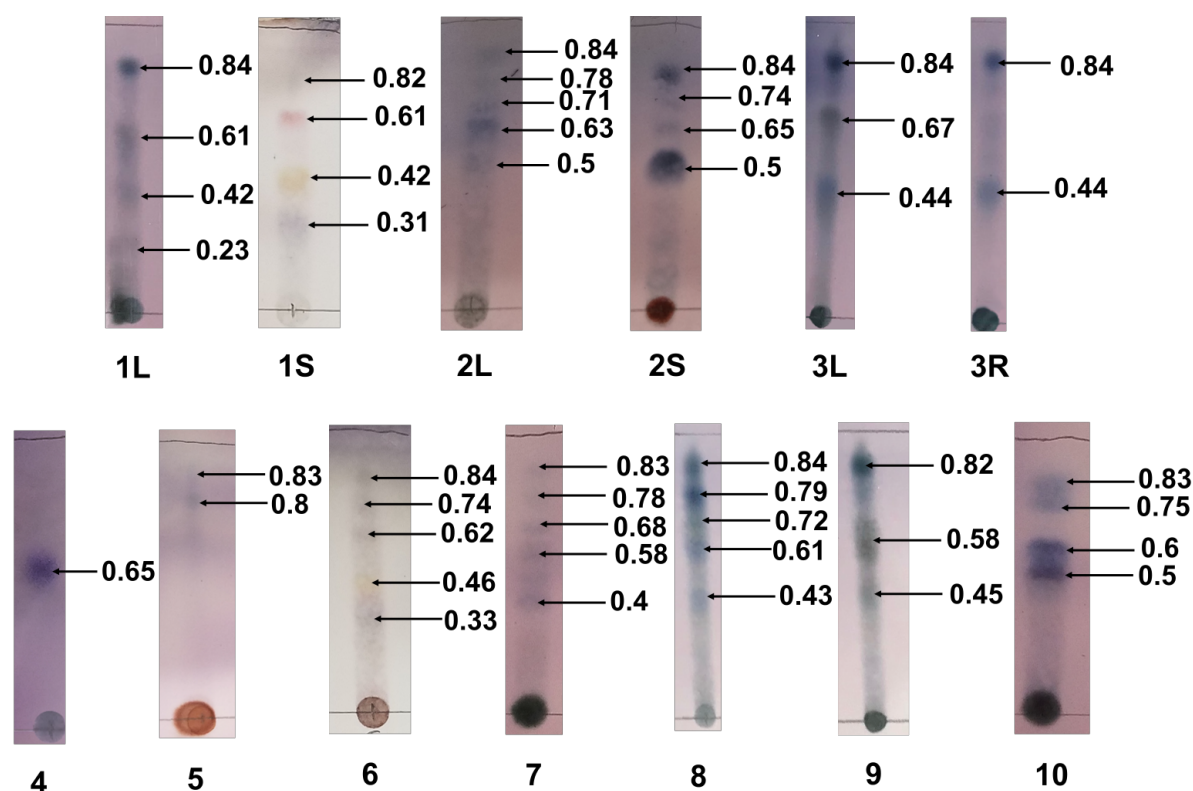
**Fig. 1 TLC profiles of the described plant species obtained using a hexane/ethyl acetate (3:1) mobile phase and revealed with anisaldehyde reagent. Rf values are also indicated**

*Azadirachta indica* (1L: leaves, 1S: seeds), *Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), *Moringa oleifera* (7), *Talinum fruticosum* (8), *Tridax procumbens* (9), and *Xylopi aethiopica* (10)

## Fig. 2 HPLC profiles of the described plant species

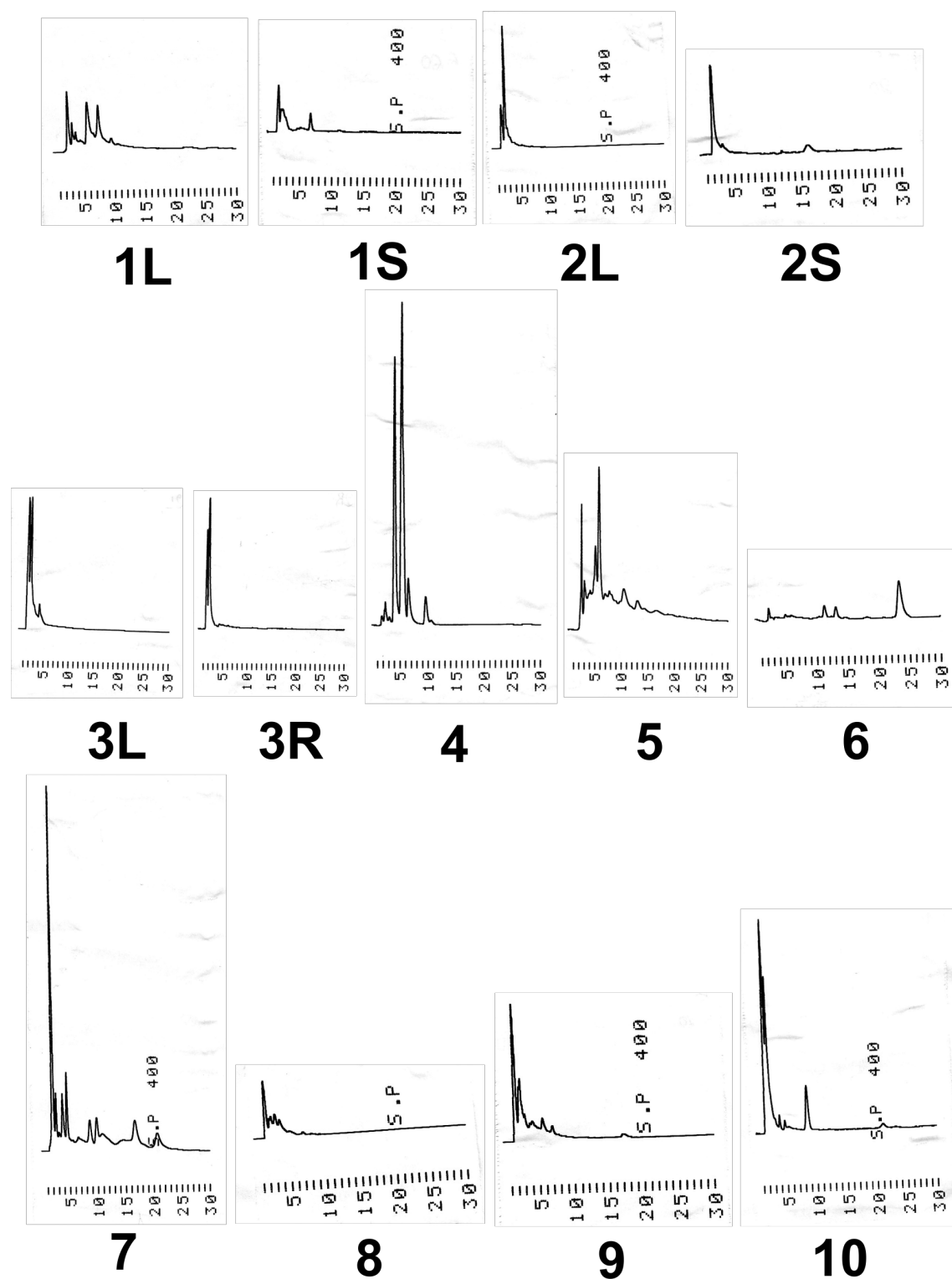
*Azadirachta indica* (1L: leaves, 1S: seeds), *Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), *Moringa oleifera* (7), *Talinum fruticosum* (8), *Tridax procumbens* (9), and *Xylopi aethiopica* (10)

## Figure Legends



**Fig. 1 TLC profiles of the described plant species obtained using a hexane/ethyl acetate (3:1) mobile phase and revealed with anisaldehyde reagent. Rf values are also indicated**

*Azadirachta indica* (1L: leaves, 1S: seeds), *Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), *Moringa oleifera* (7), *Talinum fruticosum* (8), *Tridax procumbens* (9), and *Xylopiya aethiopica* (10)



**Fig. 2 HPLC profiles of the described plant species**

*Azadirachta indica* (1L: leaves, 1S: seeds), *Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), *Moringa oleifera* (7), *Talinum fruticosum* (8), *Tridax procumbens* (9), and *Xylopia aethiopica* (10)

## Supplementary data

**Medicinal uses, thin-layer chromatography and high-performance liquid chromatography profiles of plant species from Abomey-Calavi and Dantokpa Market in the Republic of Benin**

Authors: Godfried Dougnon and Michiho Ito\*

*Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan*

\* Corresponding author:

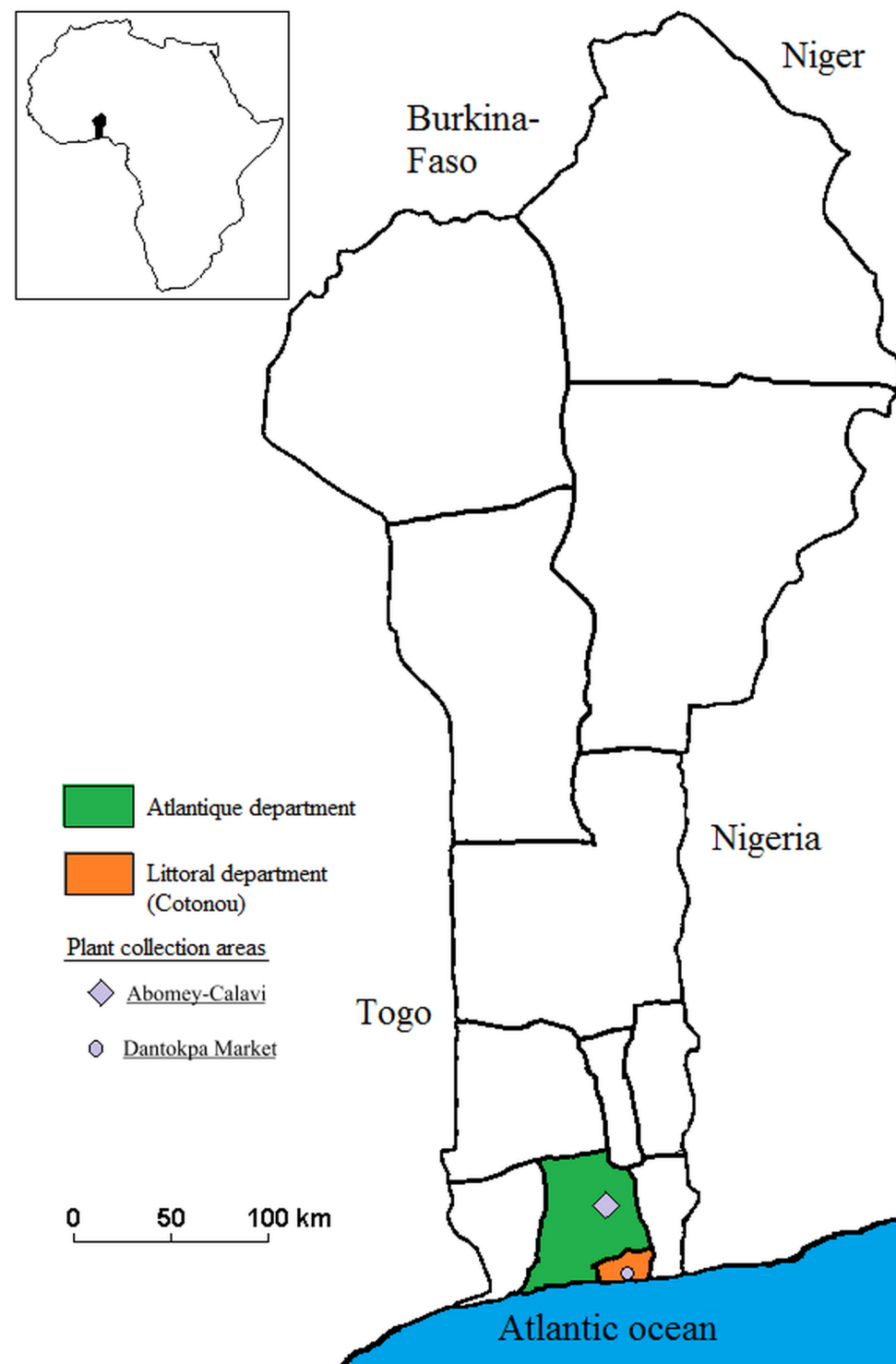
E-mail: [michihoi@pharm.kyoto-u.ac.jp](mailto:michihoi@pharm.kyoto-u.ac.jp)

Tel: +81-75-753-4506

## Contents

<b>Fig. S1</b> Study area .....	<b>3</b>
<b>Fig. S2</b> Pictures of the plant species collected in Abomey-Calavi .....	<b>4</b>
<b>Fig. S3</b> Pictures of the plant species bought in Dantokpa Market .....	<b>5</b>
<b>Fig. S4</b> TLC profiles of the described plant species obtained using a hexane/ethyl acetate (3:1) mobile phase and observed at UV 365 nm. $R_f$ values are indicated .....	<b>6</b>
<b>Fig. S5</b> TLC profiles of the described plant species obtained using a hexane/ethyl acetate (3:1) mobile phase and observed at UV 254 nm. $R_f$ values are indicated .....	<b>7</b>





**Fig. S1 Study area**

The Republic of Benin is located in West Africa. The Atlantique and Littoral departments are located in southern Benin. Abomey-Calavi is shown by a diamond and is located in the Atlantique department. The Littoral department is represented by Cotonou, where Dantokpa Market is located (indicated by a circle)



1L



1S



3L



3R



7



8



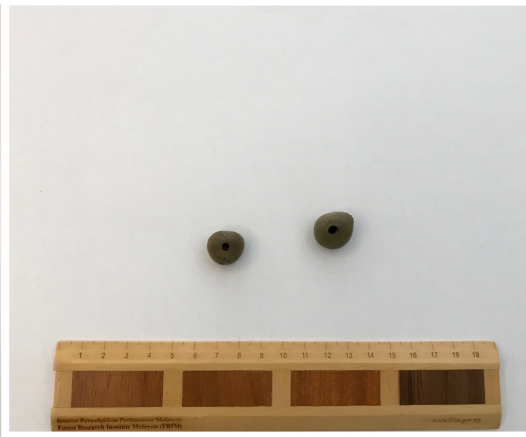
9

**Fig. S2 Pictures of the plant species collected in Abomey-Calavi**

*Azadirachta indica* (1L: leaves, 1S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Moringa oleifera* (7), *Talinum fruticosum* (8), and *Tridax procumbens* (9)



2L



2S



4



5



6

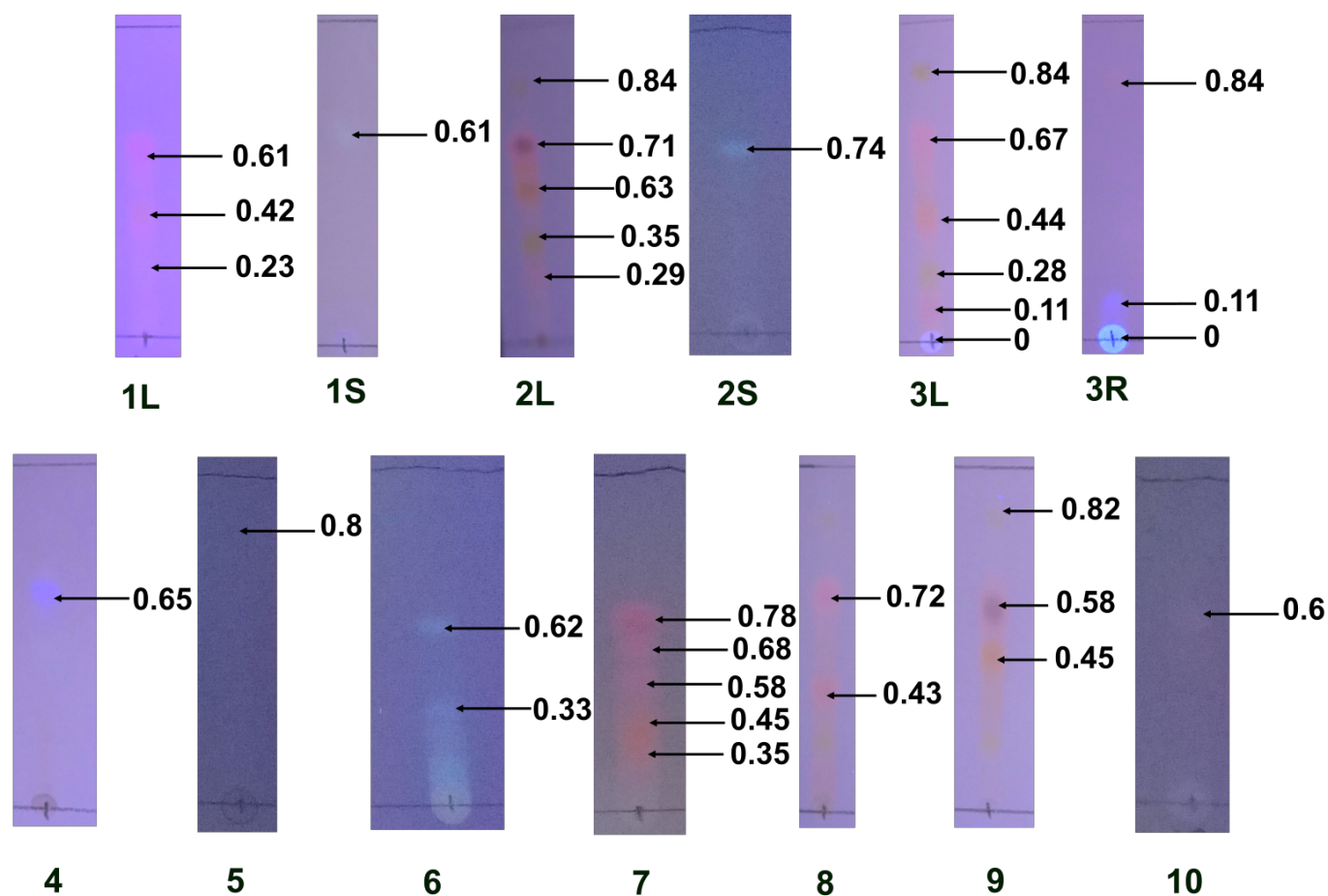


10

**Fig. S3 Pictures of the plant species bought in Dantokpa Market**

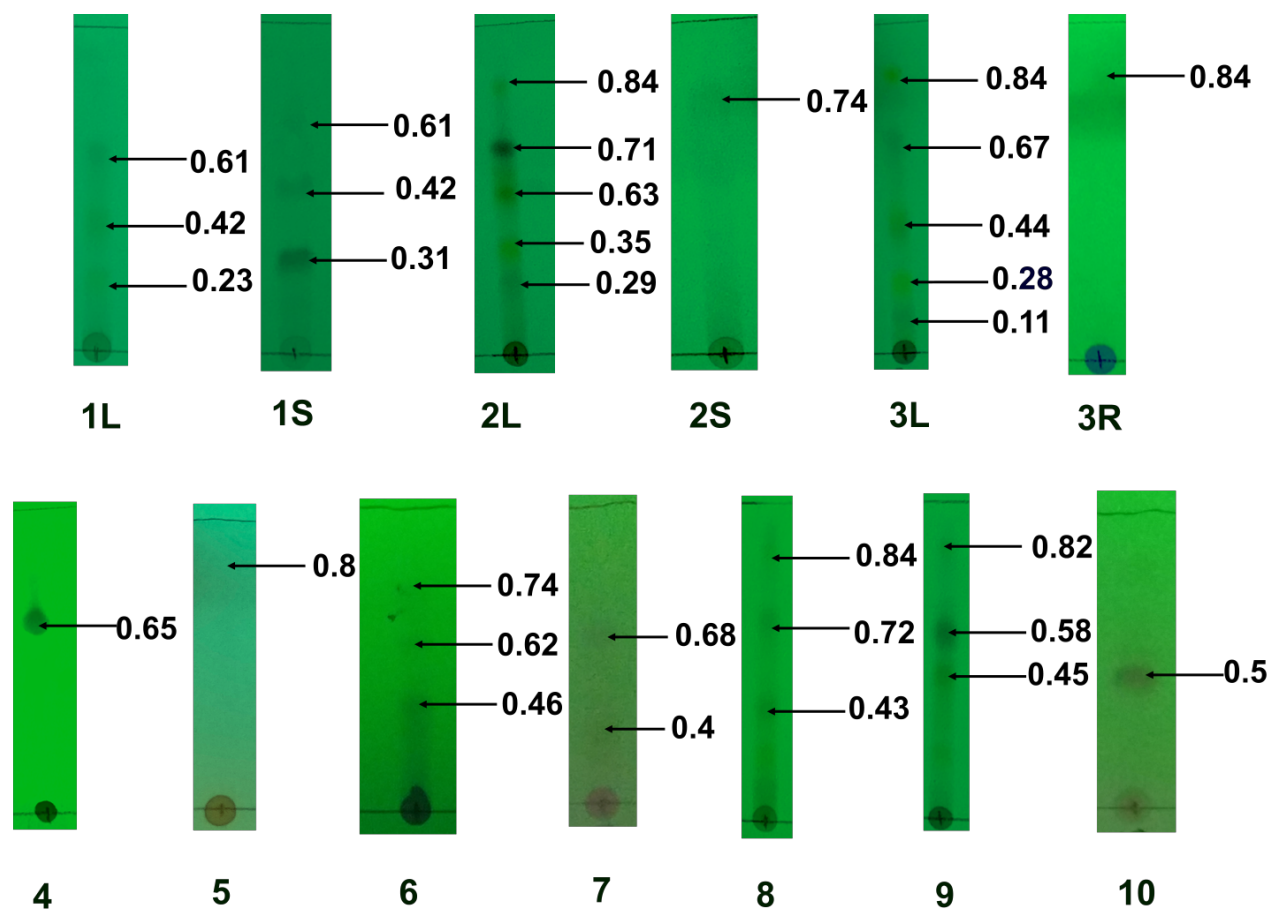
*Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), and *Xylopia aethiopica* (10)





**Fig. S4 TLC profiles of the described plant species obtained using a hexane/ethyl acetate (3:1) mobile phase and observed at UV 365 nm.  $R_f$  values are indicated**

*Azadirachta indica* (1L: leaves, 1S: seeds), *Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), *Moringa oleifera* (7), *Talinum fruticosum* (8), *Tridax procumbens* (9), and *Xylopi aethiopica* (10)



**Fig. S5 TLC profiles of the described plant species obtained using a hexane/ethyl acetate (3:1) mobile phase and observed at UV 254 nm.  $R_f$  values are indicated**

*Azadirachta indica* (1L: leaves, 1S: seeds), *Caesalpinia bonduc* (2L: leaves, 2S: seeds), *Catharanthus roseus* (3L: leaves, 3R: roots), *Garcinia kola* (4), *Khaya senegalensis* (5), *Monodora myristica* (6), *Moringa oleifera* (7), *Talinum fruticosum* (8), *Tridax procumbens* (9), and *Xylopia aethiopica* (10)